MICROSURGERY TECHNIQUE ON INSECTS

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FIGURES NOT INCLUDED IN TRANSLATION

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[Following is the translation of an article by N. A. Tamarina, Combined Laboratory of the Biology-Pedology Faculty, Moscow State University, published in the Russian-language periodical Zoologicheskiy Zhurnal (Zoological Journal), 1963, XLII, issue 8, pages 1260-1264. Translation performed by Sp/6 Charles T. Ostertag Jr.]

Experimental trends, which are being developed more extensively in entomology, in many cases require the performance of delicate operations. This is necessary during biochemical, physiological, and experimental-ecological investigations when it is necessary to extract individual organs, transfer them, transfuse hemolymph, etc. Methods for the manual dissection of insects have been described by Ye. N. Pavlovskiy (1957). In the last ten years methods of intravitam dissection have been developed by a number of authors. These can be called microsurgery. These methods and also the set of equipment are presented more fully in the report of Novak (V. A. I. Novak, 1960), which is devoted to the study of the hormonal organs of insects. The present article gives an account of the technique used by us in the practice of insect microsurgery while using new instruments and attachments as well as those already known. Some of these are suggested by us. Others, namely the stand for fixing and constricting the object being operated on, and the new designs of micropipettes and microinstruments on the basis of surgical tweezers , have been suggested by A. B. Lange (see page 1257).

The combination of equipment and single moments of work decided the success of the work. This guaranteed the accuracy of work and the rapid and successful conduct of the operations.

The microsurgical stage which we have proposed (fig 1) and on which all the procedures are carried out serves as the technical base for the method. The stage is prepared in the following manner. From plexiglas, wood or other material a base is made in the form of a cuvette without the forward wall. The cuvette is filled up with a mixture of wax with paraffin. The right rear

portion of the stage serves as a wax bath for the preparation (1). Here the extraction of the organs and tissues of the insects is performed. In the left portion of the stage the wax film is made deeper and a black or white plate is laid on it. Somewhat higher above it in the wax covering, a slide (2) with a recess (3) is sealed in. The slide is attached in such a way that the recess is located in the forward left corner of the stage. The disarticulated organ is transferred to the slide for checking its wholeness, thorough separation from adjoining tissues, and for the execution of other analogous procedures. The organ is transferred into the recess if its temporary storage is required, for example, when for the subsequent operation it is necessary to prepare several organs. The black or white plate under the dissecting glass serves as a background. In the right front portion of the stage is located the stand (4) for fixation and constriction of the insect being operated on. The insect is fixed on the plexiglas strip with the help of an elastic synthetic tape in which a window has been cut which limits the operating field. The elastic band makes it possible to reliably and softly fix the insect being operated on in any position without injuring it. The implantation of organs, injections of liquids, etc. are performed on the stand. If insects are being operated on which are rich in hemolymph, for example, fly larvae, it is recommended to squeeze out the hemolymph from the part being operated on in order to avoid its outflow. The described device permits this to be done easily.

All the operations are carried out under a binocular. The microsurgical stage is fastened to the dissecting guide (fig 2) and at a stated moment any necessary portion of it can be rapidly and accurately introduced into the field of vision with the help of screws.

The object is illuminated with an ordinary condenser (OI-19 and others) to which a water filter is attached in order to avoid overheating.

During the operation the arms of the experimenter lie on the arm rests (fig 2, 3).

In front of the microsurgical stage a micropipette (fig 2,4) for the injection of liquids and implantation of organs is attached to the stage of the binocular. The micropipette designed by A. B. Lange has been successfully approved by us.

The pipette is attached to the stand which permits it to be shifted and fixed in any position and also to put it in a non working position. Conveying the object to the pipette and introducing the pipette into the object is carried out by the movement of the microsurgical stage with the help of the dissecting guide screw.

Rests for instruments are placed on both sides of the binocular. On them are respectively placed the instruments necessary during the given operation for working with the left and right hand. A spare set of instruments is placed along side for a rapid change of any of them in the event of a breakdown.

The set of microsurgical equipment includes dissecting instruments in addition to the optics, microsurgical stage and micropipette. The set of instruments (fig 3) includes delicate dissecting needles made from entomological pins (1) and insect pins (2), a scalpel (3) made from a sliver of a safety razor blade, delicate scissors (4) (the scissors are of the Bekker or some other design), tweezers (5) with sharply pointed ends, microforceps (6), a platinum loop (7), glass rods (8) of various size, metal and glass spatulas, and an electric cautery (9).

A general view of the working table is shown in figure 4. The equipment presented on it was used by us, particularly when working with the hormone organs of insects.

As an example, we will describe an operation on Calliphora erythrocephala larvae when transplanting corpora allata. This organ in the larvae of higher Diptera is part of the hormone complex named the Weismann's ring which is located in front of the brain around the aorta.

Before the operation the larva-donor should be thoroughly washed. Having been taken from the nutrient substrate, it is washed in water, then rapidly dipped in 96° alcohol and rinsed in a great amount of distilled water. After this the larva is anesthetized.

The anesthetized larva is placed in the wax bath of the microsurgical stage, fastened here with entomological pins with the ventral side up and flushed with a physiological solution. In the area of the III thoracic segment above the brain, a T-shaped cut of the cuticle is made with the Bekker scissors. With the thin dissecting needles the nerves and tracheae going to the brain are cut transversely, excluding those to which the Weismann's ring is adjacent. During the operation it must be watched that the esophagus isn't harmed since this will lead to contamination of the tissues. Therefore, it is expedient to dissect the brain from one side at the site of junction of the forward and rear lobes, to free the esophagus and set it off to the side. After this, the brain is drawn out above with the fine tweezers and with the Bekker scissors the aorta is cut in front of and behind Weismann's ring, and also the tracheal cords in front of Weismann's ring. The Weismann's ring is left connected only with the brain with the help of the tracheae. This entire complex of organs is rapidly transferred with the tweezers to a drop of physiological solution located on the dissecting glass of the microsurgical stage

which by means of the screws on the dissecting guide is moved into the field of vision. First, the brain is detached with dissecting needles, then with great accuracy the upper part of Weismann's ring is cut out which includes the corpora allata surrounded by R-cells. The disarticulated part is transferred by the platinum loop into a drop of physiological solution on the slide with the recess of the microsurgical stage. If for the transplanting several c. allata are required, then the described procedure is conducted with other larvae and all the disarticulated organs are stored in the recess.

Upon completion of the disarticulation of the c. allata, the implantation of the larva-recipient begins; the latter is preliminarily rinsed in water and alcohol, just as the donor, and anesthetized. We used ether narcosis and the water narcosis recommended by Novak. During the water narcosis the larvae are submerged for 3-4 hours in water, in ether narcosis -- in ether vapor for three minutes. The anesthetized larva is fastened to the stand of the microsurgical stage. It is placed on the stand in such a way that the cut of the elastic tape fits on the operating field of the next to last abdominal segment. In order to avoid the outflow of hemolymph it is forced out of the sector of the body being operated on. For this, it is necessary to carefully press on the operating field with a glass rod, simultaneously tightening and loosening the tape of the stand with the help of the special screw. As a result, the larva turns out to be securely attached in the required position and the pressure of the hemolymph in the sector being operated on is relaxed.

With a small scalpel, made from a razor blade, a small incision is made in the cuticle. Several crystals of penicillin or streptomycin are put into the wounds with a glass rod, after which the implantation of the organs begins. The glass recess where the implants are located is brought into the field of vision and one of them is taken with the platinum loop and transferred to the edge of the wound. Introduction of the organ inside is performed with a glass rod. In this manner the necessary organs are implanted in turn.

If the organs being implanted are very small they are introduced into the cavity of the larva with the help of a micropipette. In this case with the screws of the dissecting guide and micropipette stand, the end of the latter is led up to the organ lying in the recess which is then drawn in by the pipette. After this, the area with the wound is led to the end of the pipette. The edge of the wound is carefully raised a little with fine tweezers and the pipette is introduced into the body cavity.

Upon conclusion of the operation the wound is dried lightly with filter paper and usually doesn't require any further treatment since thrombosis readily sets in. If this doesn't happen, it must be sealed with paraffin. A small fragment of paraffin is placed on the end of the electric cautery. After

heating, the paraffin forms a drop. When the drop comes in contact with the wound, the latter is sealed.

The described technique makes it possible to perform other operations of a various nature. For some of these the design of the microsurgical stage and different instruments may be somewhat modified.

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[The following English summary appears with the Russian original.]

The combination of the equipment and single moments of work are described during microsurgical operations on insects (removal of organs, their transplantation, hemolymph transfusion, etc.). The construction of the microsurgical table suggested by the author is described. Some microsurgical procedures with the application of previously known and new instruments and devices are demonstrated on an operation on Calliphora erythrocephala larvae when transplanting corpora allata.

Fig. 1. Microsurgical stage. 1 - wax bath, 2 - dissecting glass, 3 - glass with recess, 4 - stand for fixation and constriction of insect, 5 - screw for fixing the stand, 6 - screw for tightening the tape.

Fig. 2. Microsurgical attachments mounted on the stage of a binocular. 1 - microsurgical stage, 2 - dissecting guide, 3 - arm rests, 4 - micropipette on stand.

Fig. 3. Set of microsurgical instruments.
1 - dissecting needle made from an entomological pin, 2 - dissecting needle made from insect pins, 3 - scalpel made from a sliver of a blade from a safety razor, 4 - Bekker's scissors, 5 - tweezers with sharply pointed ends, 6 - microforceps, 7 - platinum loop, 8 - glass rod, 9 - electric cautery.

Fig. 4. General view of working table.
1 - binocular microscope with microsurgical stage and micropipette, 2 - condenser with water filter, 3 - rests with microsurgical instruments, 4 - electric cautery, 5 - rack with spare set of instruments, 6 - flask for ether narcosis, 7 - flask with alcohol for treatment of object, 8 - vessel with distilled water, 9 - weighing bottles with physiological solution, distilled water, and alcohol, 10 - weighing bottle with penicillin.